



## LC/MS

## Natural Food Colorants

## Analysis of Natural Food Colorants by Electrospray and Atmospheric Pressure Chemical Ionization LC/MS

### Introduction

Many kinds of natural colors are used in beverages, jellies, and candies. In many countries, food regulations have been recently revised to cover natural colorants to the same degree as synthetic ones. Accordingly, it has become necessary to develop reliable analytical methods for various natural colorants in food. In this study, LC/MS methods using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) were developed to identify major pigments in four natural colorants: red cabbage, paprika, Monascus, and lac.

### Experimental

Paprika and Monascus colorants were dissolved in acetone; the other colorants were dissolved in deionized water. Each colorant was filtered through a 0.2- $\mu$ m filter. A 10- $\mu$ l portion was injected into the system, which consisted of an Agilent 1100 Series binary pump, thermostatted column

compartment, vacuum degasser, autosampler, and LC/MSD. The LC/MSD used either an ESI or APCI source. Complete system control and data handling were done on the Agilent ChemStation for LC/MS. Operating conditions were optimized for each sample.

### Results and discussion

#### *Red cabbage colorant.*

Figure 1 shows the structure of seven major pigments of red cabbage. The pigments share the basic cyanidin 3-diglucoside structure with differing R<sub>1</sub> and R<sub>2</sub> groups. Figure 2 shows the total ion chromatogram (TIC) and extracted ion chromatograms (EIC) of red cabbage pigments. Although every major pigment can be chromatographically separated using 10% formic acid in the mobile phase, the high acid concentration reduces sensitivity. Therefore, 1% formic acid was used in this study. The EICs show the separation of the pigments based on their main ion (base peak).

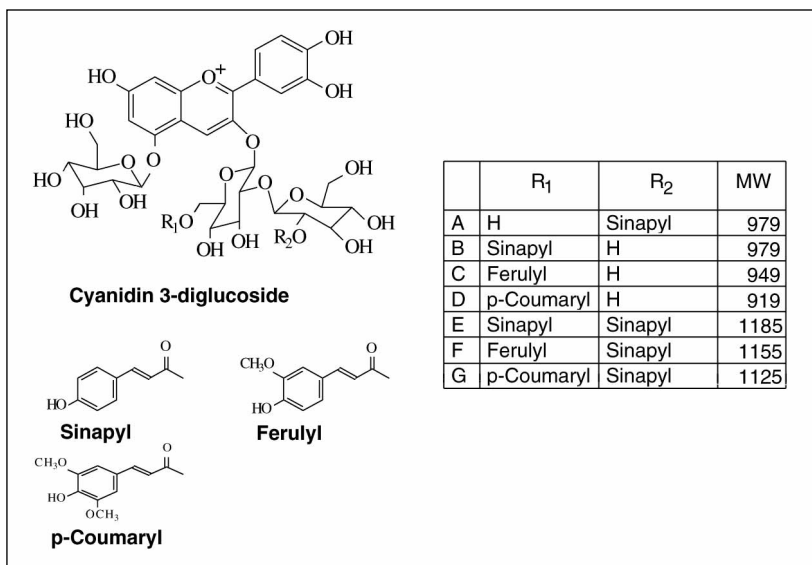


Figure 1. The structure of major pigments in red cabbage colorant.

## Natural Food Colorants

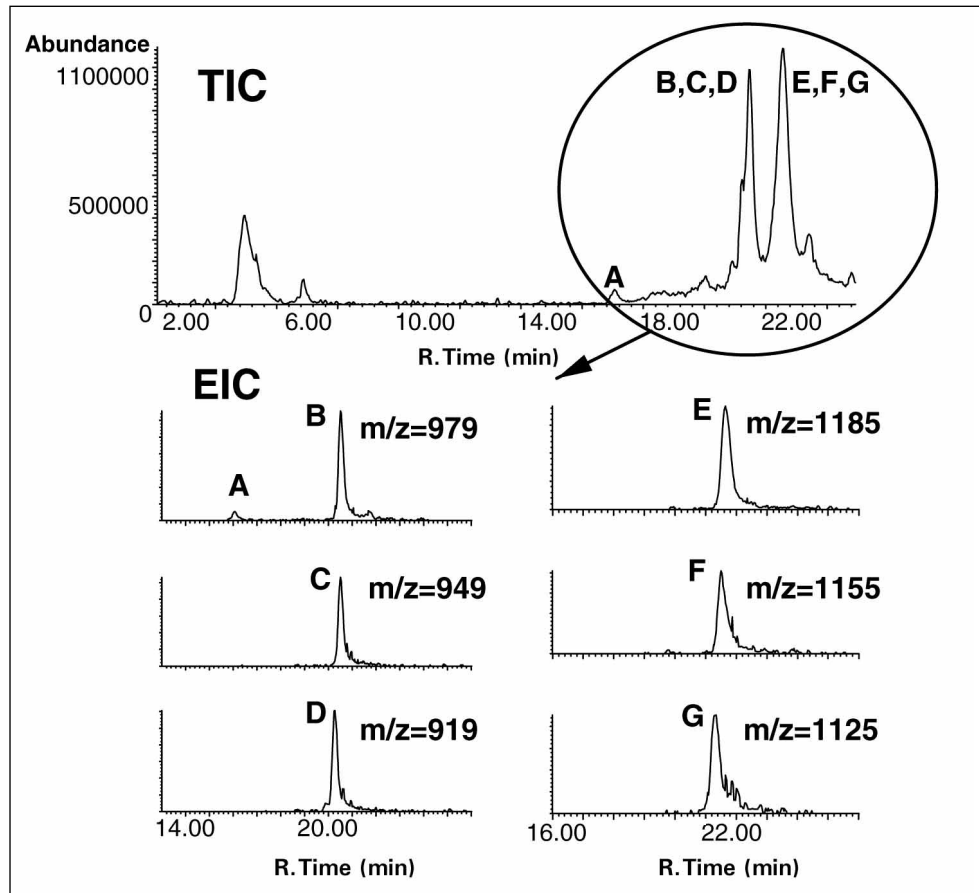
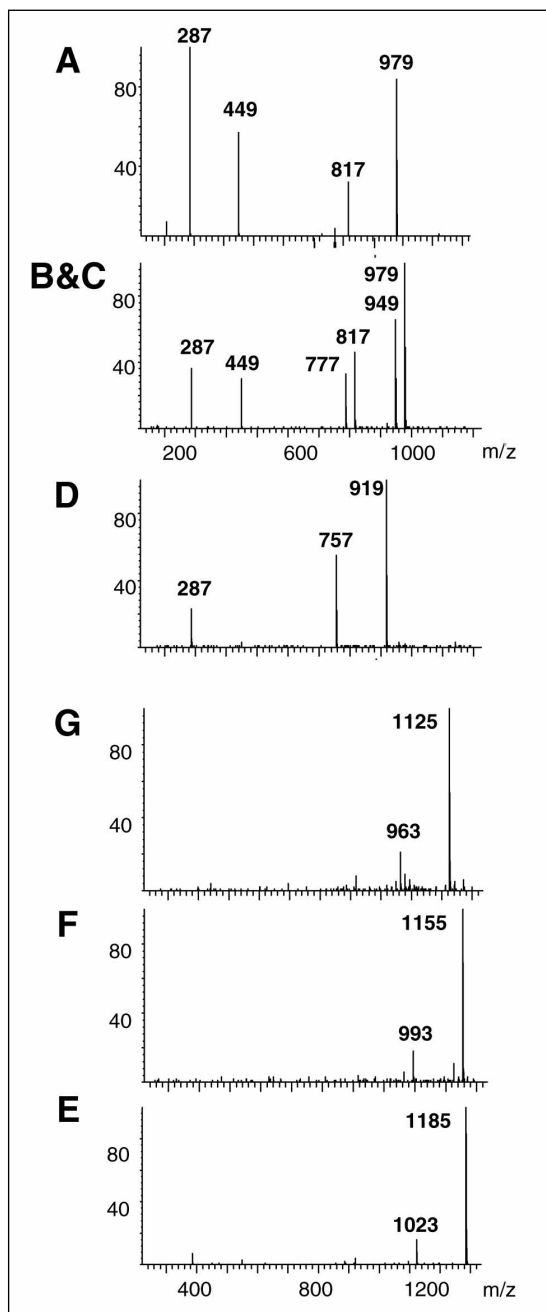


Figure 2. Total and extracted ion chromatograms of red cabbage colorant.



**Figure 3. Mass spectra of major pigments in red cabbage colorant.**

## LC conditions

Column: 250 x 2.1 mm Inertsil ODS3, 5  $\mu$ m  
 Mobile phase: A = 1% formic acid  
 B = acetonitrile  
 Gradient: Start with 5% B  
 At 30 min 50% B  
 Flow rate: 0.2 ml/min  
 Column temp: 40° C  
 Injection vol: 10  $\mu$ l

## MS Conditions

Source: ESI  
 Ion mode: positive  
 Vcap Voltage: 4000 V  
 Nebulizer: 50 psig  
 Drying gas flow: 10 l/min  
 Drying gas temp: 350° C  
 Corona: 4  $\mu$ A  
 Vaporizer temp: 350° C  
 Scan range: 100-1200 amu  
 Step size: 0.1  
 Peak width: 0.15 min  
 Time filter: on  
 Fragmentor: 200 V

Figure 3 shows the mass spectra of the seven major pigments in red cabbage colorant. For these pigments, the singly charged molecular ion is observed rather than the more typical  $[M+H]^+$  ion, because the cyanidin group already has a positive charge on an oxygen. In-source collision-induced dissociation (CID) can be used to generate fragment ions to provide structural confirmation. Using CID, mass spectra of these pigments show common fragments corresponding to the loss of a glucose, as well as cyanidin ( $m/z$  287) and cyanidin 3-glucoside ( $m/z$  449) ions.

## *Monascus colorant*

*Monascus* contains six major pigments; their structures are shown in Figure 4. Four pigments were identified from the mass spectra of major peaks in the TIC. See Figure 5.

## Natural Food Colorants

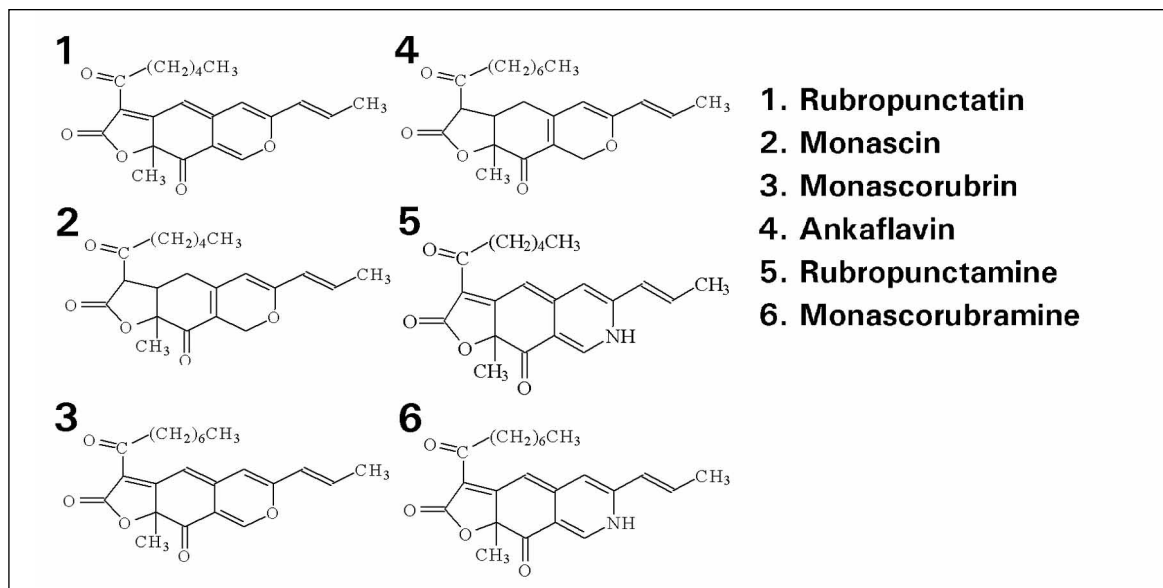


Figure 4. The major pigments of *Monascus* colorant.

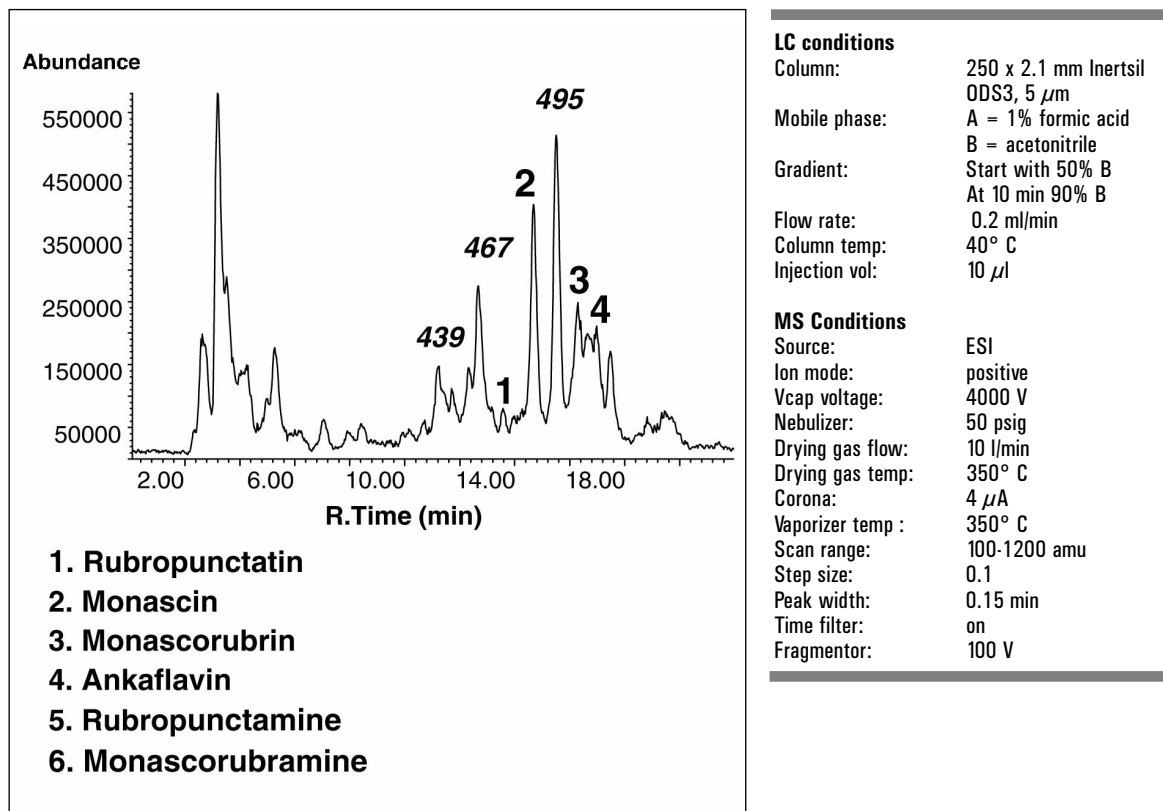
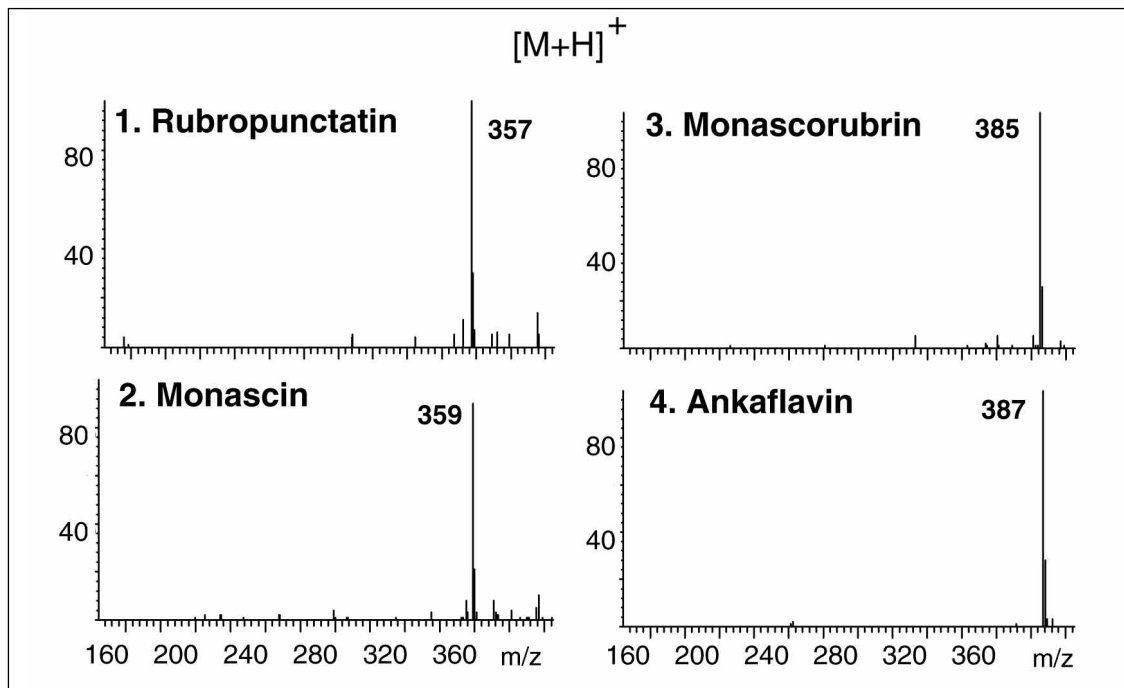


Figure 5. The total ion chromatogram of *Monascus* colorant.



**Figure 6. Mass spectra of major pigments in *Monascus* colorant.**

Three major peaks with base peaks at  $m/z$  439, 467, and 495 were not identified. Figure 6 shows the mass spectra of the identified pigments. Protonated molecular ions  $[M+H]^+$  were observed for the four identified pigments.

### *Paprika color*

Capsanthin and the mono- and di- esters of capsanthin with fatty acids are known as the major pigments in paprika colorant. See Figure 7. Two monoesters and five diesters of capsanthin were identified in the paprika colorant analyzed in this study. See Figure 8.

The protonated molecular ions  $[M+H]^+$  were observed for every major pigment. See Figure 9. However, with the exception of capsanthin monoecicosanoate, the intensity of these ions was very low. Except for capsanthin monoecicosanoate, the pigments show fragment ions resulting from the loss of one or two fatty acid fragments. A common fragment ion was observed at  $m/z$  567 in the mass spectra of these pigments.

## Natural Food Colorants

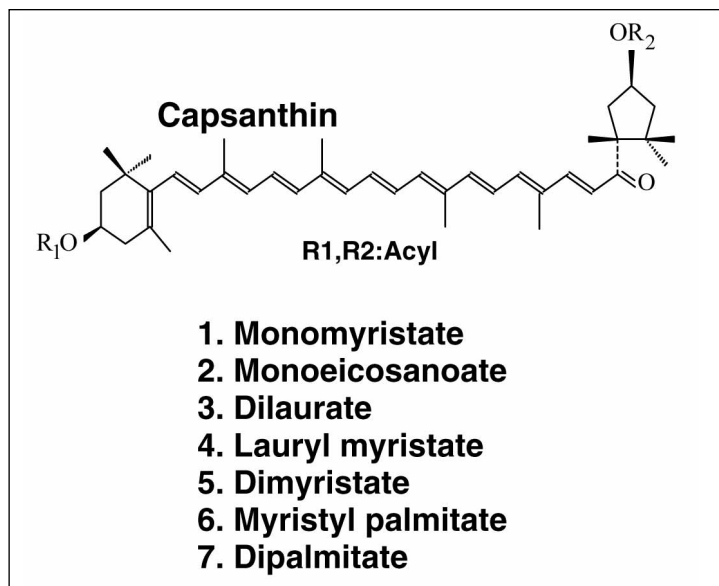


Figure 7. The structure of major pigments of paprika colorant.

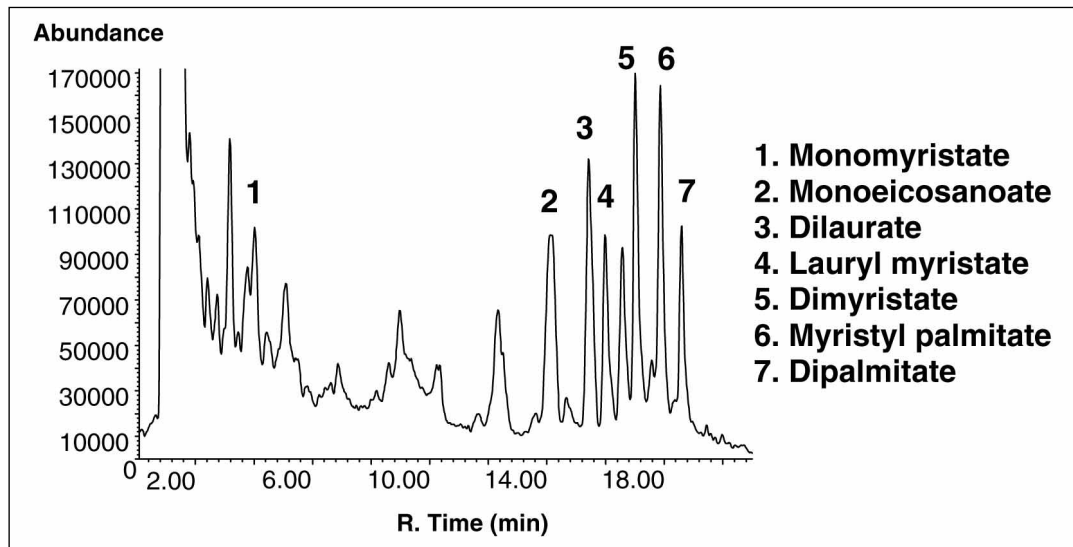
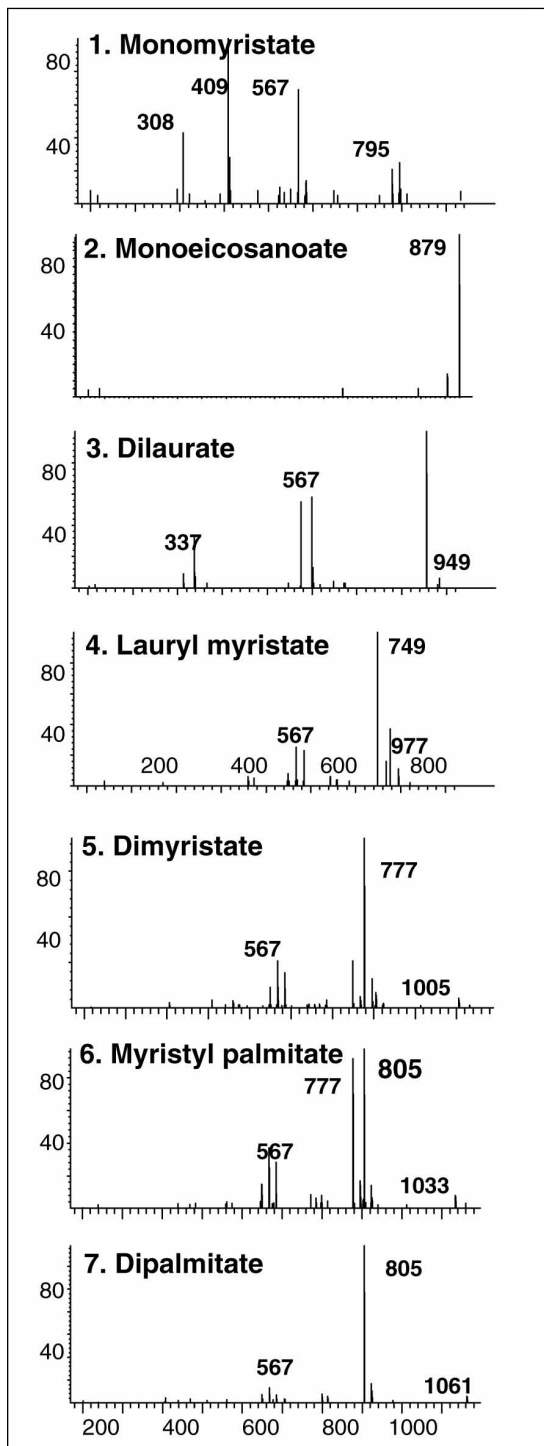


Figure 8. The total ion chromatogram of paprika colorant.



**Figure 9. Mass spectra of major pigments in paprika colorant.**

## LC conditions

Column: 250 x 2.1 mm Inertsil  
ODS3, 5  $\mu$ m  
Mobile phase: A = acetone  
B = methanol  
Gradient: Start with 10% B  
At 10 min 90% B  
Flow rate: 0.2 ml/min  
Column Temp: 40° C  
Injection vol: 10  $\mu$ l

## MS Conditions

Source: APCI  
Ion mode: Positive  
Vcap voltage: 4000 V  
Nebulizer: 50 psig  
Drying gas flow: 5 l/min  
Drying gas temp: 350° C  
Corona: 4  $\mu$ A  
Vaporizer temp: 350° C  
Scan range: 100-1200 amu  
Step size: 0.1  
Peak width: 0.15 min  
Time filter: On

## Lac colorant

Figure 10 shows the structure of the major pigments in lac colorant. Laccaic acids A, B, C are known as the major pigments in lac colorant. These compounds have the same basic anthraquinone structure but with different R groups. Three major peaks were detected in the TIC. See Figure 11. Although laccaic acids A, B, and C were identified, A and B could not be separated.

Figure 12 shows the mass spectra of two peaks, laccaic acid C and a combination of laccaic acids A and B. The deprotonated molecular ions were observed at m/z 495, 536, and 538. Fragment ions resulting from the loss of carbon dioxide were observed at m/z 451, 492, and 494.

## Natural Food Colorants

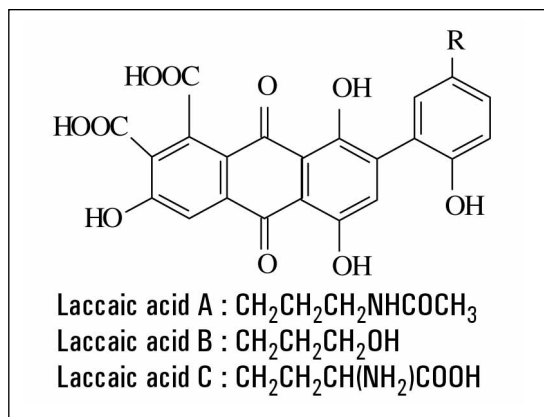


Figure 10. The structure of major pigments of lac colorant.

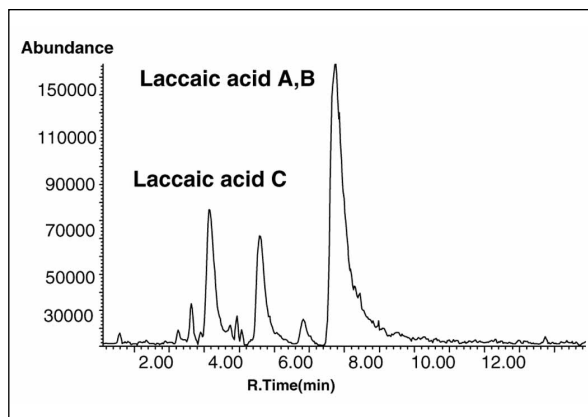


Figure 11. The total ion chromatogram of lac colorant.

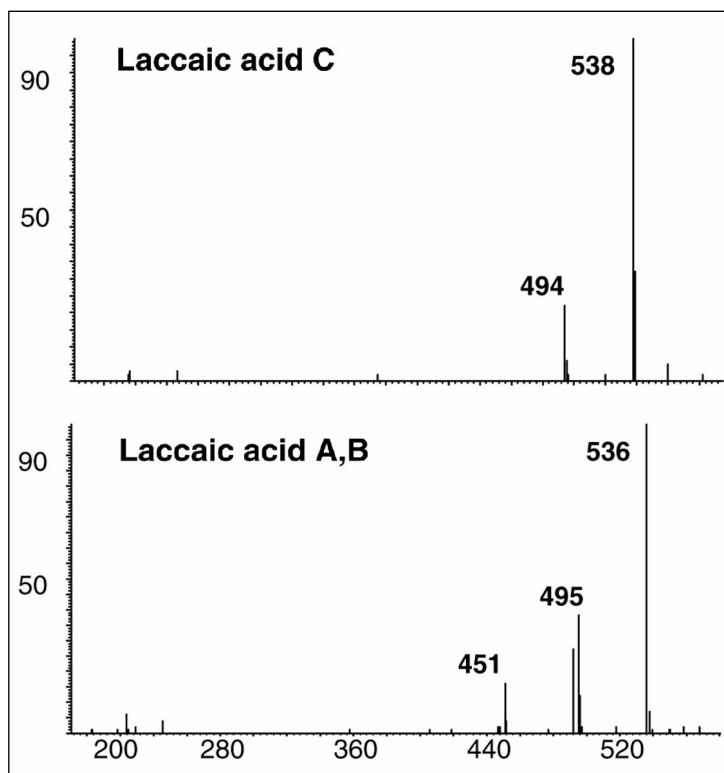


Figure 12. Mass spectra of the major pigments in lac colorant.

### LC conditions

Column: 250 x 2.1 mm Inertsil  
 ODS3, 5  $\mu\text{m}$   
 Mobile phase: 30% acetonitrile in 5  
 mM dibutylamine,  
 isocratic  
 Flow rate: 0.2 ml/min  
 Column Temp: 40° C  
 Injection vol: 10  $\mu\text{l}$

### MS Conditions

Source: ESI  
 Ion mode: Negative  
 Vcap voltage: 4000 V  
 Nebulizer: 50 psig  
 Drying gas flow: 10 l/min  
 Drying gas temp: 350° C  
 Scan range: 100-1200 amu  
 Step size: 0.1  
 Peak width: 0.15 min  
 Time filter: On  
 Fragmentor: 100 V





## Natural Food Colorants

---

### Conclusion

Four commercial natural colorants were analyzed using ESI and APCI-LC/MS. The MS data provided molecular weight information and some structural information for the major pigments.

*Masahiko Takino* is an applications chemist at Yokogawa Analytical Systems, Inc.

Windows NT<sup>®</sup> is a U.S. registered trademark of Microsoft Corporation.

Windows<sup>®</sup> is a U.S. registered trademark of Microsoft Corporation.

Agilent Technologies shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance or use of this material.

Information, descriptions and specifications in this publication are subject to change without notice.

Copyright © 1998  
Agilent Technologies, Inc.  
All rights reserved. Reproduction and adaptation is prohibited.

Printed in the U.S.A. April 2000  
(23) 5968-2979E